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Evaluation of Antidiabetic Activity of *Nauclea Latifolia* Chloroform Root Extract in Normal and Alloxan-Induced Diabetic Rats

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Abstract: *This study was carried out to evaluate antidiabetic activity of chloroform extracts of the root of Nauclea latifolia in both normal and alloxan-induced diabetic rats. Phytochemical analysis of the root extracts of Nauclea latifolia revealed the presence of bioactive compounds such as alkaloids, tannins, saponins, flavonoids, cardiac glycosides, steroids and terpenoids capable of eliciting biochemical and pharmacological actions and may be responsible for it significant antidiabetic action in alloxan-induced diabetic rats. This is an indication that chloroform is suitable in extracting the active phytochemicals present in the plant. The chloroform extracts at 200mg/kg body weight and Glibenclamide at 1mg/kg body weight showed significant glycaemic reduction of 59.4% and 66.4% in alloxan-induced diabetic rats and on the other hand showed only 18.5% and 60.5% glycaemic reduction in non-diabetic rats respectively. These findings suggest that chloroform extracts of the root of Nauclea latifolia posses antidiabetic potentials that validate its use in the management of diabetes locally.*

Key words: *Nauclea latifolia chloroform root extract, antidiabetic activity, glycaemic reduction, alloxan-induced diabetic rats and phytochemical constituents.*

I. Introduction

Antidiabetics are natural or synthetic agents used for the management of diabetes, which is a common metabolic disorder characterized by hyperglycaemia caused by absolute or relative deficiency of insulin. Adult on-set (Type 2) diabetes accounts for about 90 percent of cases. Some diabetic patients can be managed on diet alone; most require oral hypoglycaemic drugs and/or insulin. Worthy of note is the efforts that have been made to discover new antidiabetic compounds from various sources; one of such resources is the folk medicine. Studies have been carried out on the hypoglycaemic activity of the aqueous extract of the leaves of *Nauclea latifolia* plant in alloxan-induced diabetic rats (Gidado *et al.*, 2005). The use of insulin and/or oral hypoglycaemic drugs is not without short comings and side effects (Rang and Dale, 1991). These short comings and side effects led to the search for alternative remedies which may produce similar degree of efficacy. It is noteworthy that World Health Organization (WHO) encouraged research on hypoglycaemic agents of plant origin and this has greatly motivated research in this area. In the last few decades many plants and plant products have been reported to have antidiabetic property (Handa *et al*., 1989; Jia *et al*., 2003). Therefore seeking remedies for human ailments from the environment has formed the basis for therapeutics (Potier et al., 1990).

Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties (Serrentino, 1991). Aqueous extract of the leaves of the plant has been as remedy for diabetes mellitus in Northern Nigeria (Gidado *et al*., 2005).

It has been reported that *Nauclea latifolia* is one of the six most prescribed medicinal plants among the Igede people of Benue State (Igoli, *et al*., 2005). The plant has also been reported to have antihypertensive and laxative activities (Akpanabiatu *et al*., 2005). Therefore, it is on this basis that this investigation was carried out to evaluate the antidiabetic potentials of the chloroform root extract of *Nauclea latifolia*.

II. Materials and Methods

2.1 Test Plant

The root of *Nauclea latifolia* was collected in June 2010 from the farmland behind Ex-Service Officers Estate, Kurudu Abuja, Nigeria. The plant was identified by Dr P.O Egwumah of the College of Forestry, Federal University of Agriculture Makurd, Benue State, Nigeria.

2.2 Experimental Animals

Thirty Swiss albinos mature male rats of weight range (150-200g) obtained from National Veterinary Research Institute Vom, Plateau State were used for the study. The rats were allowed to acclimatize to the laboratory conditions (room temperature, 12-hour light/dark cycle) for seven days. The rats were housed, two in a cage. They were fed with a commercial feed (mice cubes) prepared by ECWA feed Jos, Plateau State, Nigeria and water ad libitum.

2.3 Drugs and their Sources

The following drugs were used in the study:

i. Glibenclmide (GLIBEN-J$^{(R)}$, Juhel, Nigeria) 5mg white scored caplet marked 'GLI/5' on one side and 'Juhel' on the reverse.
ii. Alloxan monohydrate (Sigma-Aldrich Company Limited, United Kingdom).

2.4 Extraction Procedure

The root of *Nauclea latifolia* collected was sun dried and pulverized by pounding. One hundred grams of the powdered root of *Nauclea latifolia* was placed in a corked bottle, and 500 ml of solvent (chloroform) was added in the cold (cold extraction). The resulting suspension was allowed to stand in a tightly covered bottle for 48 hours at room temperature, after which was filtered using Whatman's No.1 filter paper into a round bottom flask. The flask containing the filtrate was placed in the water bath and allowed to evaporate off the extraction solvent to obtain the crude extract. The crude extract was placed in sterile sample bottles and labeled appropriately. The yield was weighed and recorded in grams.

2.5 Screening for Phytochemical Components

Phytochemical test was carried out on the extract for the following plant constituents: tannins, saponins, cardiac glycosides, alkaloids, steroids and flavonoids. Chemical tests were carried out on the chloroform extract of the root using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (2002), and Harborne (1998).

2.5.1 Test for Tannins: Small portion of extract of root was stirred with 1ml absolute methanol and $FeCl_3$ (aq) added in a test tube. A blue black precipitate is indicative of the presence of tannins.

2.5.2 Test for Saponins

i. Frothing test: Small portion of extract of root was shaken with water in a test tube. Frothing which persisted on warming is indicative of the presence of saponins.
ii. Emulsion test: Five drops of olive oil was added to 3ml of extract of root in a test tube and the mixture was vigorously shaken to form stable emulsion. Formation of stable emulsion indicates the presences of saponins.

2.5.3 Test for Flavonoids: 2ml of solution of extract was added to Magnesium chip and few drops of H_2SO_4 in a test tube. Light yellow precipitate in brown solution is indicative of flavonoids.

2.5.4 Test for Alkaloids:

i. Small portion of extract of root was dissolved in 2ml 0.1 HCl (aq) in a test tube. To this was added 2 drops of Mayer's reagent, a light yellowish white precipitate indicates the presence of alkaloids.
ii. Dragendroff's reagent, 0.85g basic bismuth nitrate in 10ml glacial acetic acid into 40ml water was added to small portion of extract of root in a test tube and the mixture was heated for 2minutes. Faint yellowish orange precipitate indicates the presence of alkaloids.

2.5.5 Test for Cardiac glycosides: Keller-Killani test: To a small portion of solution of extract in glacial acetic acid containing 2 drops of $FeCl_3$ (aq) was added 1.5ml conc. H_2SO_4. Lower concentrated H_2SO_4 layer that is colourless, with upper acetic acid layer is indicative of the presence of glycosides.

2.5.6 Test for Steroids: The extract was dissolved in 2ml of acetic anhydride and cooled ice concentrated H_2SO_4 was carefully added. A colour change from violet to blue then to green indicates the presence of steroids.

2.6 Determination of Antidiabetic Activity

2.6.1 Experimental Design

The study was carried out on non-diabetic and alloxan-induced diabetic rats. The rats were fasted for 18 to 24 hours before each experiment, and blood samples were collected from the tail of the rats.

2.6.2 Induction of Diabetes

Diabetes was induced by a single intra-peritoneal injection of 150 mg/kg of alloxan monohydrate dissolved in normal saline after an overnight fast. After two weeks, surviving rats with blood glucose more than 200mg/100ml were considered as alloxan-induced diabetic rats.

2.6.3 Non-Diabetic Rats

Fasting blood glucose concentration was first determined in overnight fasted rats following which the extract (20 mg/ml) was administered orally at a dose of 200 mg/kg via a BMI feeding tube (size 8). Blood glucose values were then estimated hourly for 4 hours. Control rats received distilled water in place of the extract. Similarly, a standard antidiabetic drug (Glibenclamide 1 mg/kg) was administered orally to another set of rats and glucose determined at the same time intervals for the same duration according to the procedure of Aderibigbe *et al*. (2001).

2.6.4 Alloxan-Induced Diabetic Rats

Chloroform extracts of the root (20 mg/ml) was orally administered to the diabetic rats at a dose of 200 mg/kg after determining their initial fasting blood glucose concentration. The blood glucose concentration was then assayed at one hour interval for 4 hours. Distilled water was administered in place of the extract for the control studies. A standard antidiabetic drug (Glibenclamide 1mg/kg) was similarly administered to the diabetic rats, blood glucose concentration determined at the same interval for the same duration.

2.6.5 Determination of Blood Glucose

Blood was collected in fluoridated tubes and the serum harvested by centrifugation at 3000 g for 10 minutes. Serum glucose concentration was determined based on the principle of Trinder (1969) using glucose oxidase kit (Randox, UK.).

Percentage glycaemic change was then calculated using the following formula:

% Glycaemic change = Glucose concentration (1, 2, 3 or 4 h) – fasting blood glucose x 100 / Fasting blood glucose.

III. Results

The phytochemical screening as shown on table 1 revealed that the chloroform extract of the root of *Nauclea latifolia* contained alkaloid, saponins, flavonoids, cardiac glycosides and steroids while tannins and terpenoids were absent.

Table 2 shows the effect of chloroform extract of *Nauclea latifolia* and Glibenclamide on blood glucose level of non-diabetic rats. The percentage glycaemic change on non-diabetic rats following administration of *Nauclea latifolia* root extract or Glibenclamide is shown in table 2. The extract caused a slight reduction in the blood glucose levels while Glibenclamide on the other hand caused a significant reduction of the blood glucose levels in non-diabetic rats. Chloroform root extract of *N. latifolia* caused an 18.5% reduction in blood glucose level compared to 60.05% observed for Glibenclamide for the same period in non-diabetic rats.

Table 3 shows the effect of chloroform extract of *Nauclea latifolia* and Glibenclamide on blood glucose level of alloxan-induced diabetic rats. The values are mean of three determinations with diabetic compared with normal control and experimental groups compared with diabetic control. Also for the diabetic rats, all the doses of the chloroform root extracts administered through the period of the experiment significantly reduced the fasting blood glucose of the alloxan-induced diabetic rats. The chloroform extract of *N.latifolia* root possess adequate antidiabetic properties as evident by the percentage glycaemic change in blood glucose levels of the diabetic rats following the administration of water, chloroform root extract of *N. latifolia* and Glibenclamide as shown in table 3.

Table 1: Phytochemical constituents of chloroform root extract of *Nauclea latifolia*

Test	Chloroform Extract
Alkaloids	+
Saponins	+
Tannins	-
Flavonoids	+
Cardiac glycosides	+
Terpenoids	-
Steroids	+

Key: +: Presence of constituent; -: Absence of constituent

Table 2: Effect of chloroform root extract of *N. latifolia* and Glibenclamide on blood glucose level of non-diabetic rats

Groups	Initial glycaemia (mg/dl)	Final glycaemia (mg/dl)	% glycaemia change
Normal control	73.12	75.32	3.00
Nuaclea latifolia Extract	72.14	58.78*	-18.52
Glibenclamide	72.16	28.83*	-60.05

Key: The values are mean of three determinations and experimental groups compared with normal control.

- Dose of *N.latifolia* extract 200mg/kg
- Dose of Glibenclamide 1mg/kg
- Experimental groups are compared with normal control
- Initial-Values before administration of extract and Glibenclaimide
- Final- Values at the end of experimental period
- $^{*}P<0.001$ Compared with normal control

Table 3: Effect of chloroform root extract of *N. latifolia* and Glibenclamide on blood glucose level of Alloxan-induced diabetic rats

Groups	Initial glycaemia (mg/dl)	Final glycaemia (mg/dl)	% glycaemia change
Normal control	73.12	75.32	3.00
Diabetic control	268.16	289.12[a]	7.52
Nuaclea latifolia Extract	267.64	108.78[b]	-59.36
Glibenclamide	288.63	96.91[b]	-66.42

Key: The values are mean of three determinations with diabetic compared with normal control and experimental groups compared with diabetic control

- Dose of *N. latifolia* extract 200mg/kg
- Dose of Glibenclamide 1mg/kg
- Diabetic control compared with normal control
- Experimental groups are compared with normal control
- Initial-Values before administration of extract and Glibenclaimide
- Final- Values at the end of experimental period
- $^{a}P<0.001$ Compared with normal control
- $^{b}P<0.001$ Compared with diabetic control

IV. Discussion

This study evaluated the phytochemical constituents of *N.latifolia* root extracts. The phytochemical tests are confirmatory of the published report of the key constituents of *Nauclea latifolia* (Iwu *et al*., 1999). Plants are known to contain secondary metabolites (active principles) these include alkaloids, saponins, terpenoids, tannins, flavonoids and glycosides that elicit physiological response. The chloroform extract contained some of the bioactive compounds such as the alkaloid, saponins, flavonoids, cardiac glycosides and steroids. This may be responsible for its antidiabetic activities. This result is in conformity with the report of Abiodun *et al*. (2007) as well as the work done by Morah (1995) who reported that *Nauclea latifolia* contains terpenes, alkaloids, glycoalkaloids and tannins, and could be the reason for it significant antidiabetic action in alloxan-induced diabetic rats. Previous studies reported that the root contains specific type of alkaloid called indole-quinolizidine alkaloids as well as glyco-alkaloid (Iwu, 1999; Shigemori *et al*., 2003). Though, the concentrations of the extracts were higher than the concentration of the Glibenclamide at 1mg/kg body weight compared to 200mg/kg body weight of the extract. It could be explained that the extract is still crude and made of complex composition of chemicals while, the standard drugs are of purer compounds. It has been reported that different parts of *N. latifolia* are prescribed for the treatment of diabetes mellitus by traditional healers. Previous study by Gidado *et al*. (2005) have evaluated this claim and confirmed hypoglycaemic effects of the plant using the ethanol extract of the leaves in alloxan-induced and streptozotocin-induced diabetic rats respectively. This present studies using chloroform extract of the root of the plant supports the antidiabetic properties of the plant. The chloroform extract of the root of *N. latifolia* caused maximal reduction of 59.4% while for Glibenclamide the maximal reduction of 66.4% was observed in alloxan-induced diabetic rats. Also Gidado *et al*., 2009 reported maximal reduction of blood glucose level of 60.77% and 69.16% for the aqueous extract of the leaves of the plant and Glibenclamide in streptozotocin-induced diabetic rats respectively. This study also revealed significant reduction of fasting blood sugar level in alloxan-induced diabetic rats following daily oral administration of Glibenclamide at a dose of 1mg/kg body weight. The antidiabetic activity of the

extract can be said to be similar with that of Glibenclamide, an oral hypoglycaemic agent that lower blood glucose in both normal and diabetic subjects (Rao and Rao, 2001).

Administration of chloroform root extract of *N. latifolia* (200mg/kg body weight) showed significant reduction of blood glucose levels of alloxan-induced diabetic rats when compared with diabetic control rats that received only distilled water. Similar effect was also observed with Glibenclamide when compared with diabetic control rats within the same period. Doses of 200mg/kg body weight of chloroform extracts of the *N. latifolia* root were administered to both non-diabetic and alloxan-induced diabetic rats daily for the period of the experiment. This result however is similar to the work of Gidado *et al.*, 2009 on antidiabetic effect of *Nauclea latifolia* leaf ethanolic extract in streptozotocin-induced diabetic rats. It is however not clear the mechanism of action of chloroform extracts of the *N. latifolia* root in lowering the blood glucose level of diabetic rats as well as the bioactive constituents responsible for the antidiabetic effect. Although, it has been reported that *N. latifolia* contain alkaloids, and some inorganic elements that could have contributed to the antidiabetic (Morah, 1995; Abreu and Pereira, 2001). Hideyuki *et al*. (2003) also reported the presence of indole alkaloids in the roots of *N. latifolia.*

V. Conclusion

The present studies shows that the plant *Nauclea latifolia* used traditionally as a therapeutic agent possess significant antidiabetic activities which can be attributed to the bioactive components present in the extracts of the plant. The observed antidiabetic effects of the plant appear to justify the ethno medicinal use as hypoglycaemic agent.

VI. Recommendations

The plant appears to be as effective as the standard drugs (Glibenclamide) used in the study to merit further comprehensive phytochemical, therapeutic, and toxicological evaluation. Since medicinal plants in Nigeria are largely uninvestigated and many of these plants do not even have baseline data of their major constituents, a bioassay guided fractionation is needed to isolate, identify, and characterize the hypoglycaemic constituent(s) of the plant using gas chromatography or mass spectral measurement techniques aiming towards application to new efficacious treatment. Future studies also need to focus on ascertaining the exact mode of action of the extract of *N. latifolia* in exerting antidiabetic effects. It may also be necessary that the pharmacology, stability and bioavailability of this product be defined. The plant should be commercially produced to benefit the populace since the cost will still be much lower than the conventional antibiotics and antidiabetics.

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